sample at 37°C. The conclusion is that they are also formed while Risedronate is retained in the bladder. No drug-related toxicity was observed in the urinary tracts or bladders of animals dosed with Risedronate.
No drug-related increases in liver weight, cytochrome P450 content or catalytic activity, or in UDP-glucuronosyltransferase activity were detected.

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Summary Review of Pharmacokinetics:

Single Oral Administration of Risedronate : (Studies G3A, G7A, G8A, G9A)

Species	Tissue	Dose (mg/ kg)	AUC (0- t) (ng/ mL * hr)	C max (ng/ mL)	T max (hr)	t 1/ 2 (hr)
Rat	Blood	0.5	10 a	7.0	0.5	0.64 b
Dog	Blood	0.5	10 a	6.0	0.45	0.93 b
		0.2	18.2	8.17	0.35	2.54 b
		0.8	79.4	37.7	0.29	36.1
Dog	Serum	4.0	654	361	0.46	12.2
		16	7623	2507	0.71	7.96
		64	40176	9517	0.92	6.79
		0.106	12.9	4.8	0.63	ND
		0.302	36.3	8.5	0.45	ND
Dog	Plasma	0.899	157	48.3	1.5	ND
		2.66	346	94.0	1.0	ND
		7.87	1999	589.7	0.58	8.4

a AUC (0- last observed sample)
b Low t estimates are reflective of the early loss of analytical sensitivity for Risedronate prior to reaching a log- linear terminal elimination phase

After single oral doses of 0.5 mg/kg ^{14}C-Risedronate in rats, blood concentrations decreased rapidly after reaching Tmax (0.5 hr) to below quantifiable levels after 2 hr, resulting in a blood concentration-time profile that could provide only rough characterization of the oral pharmacokinetics (above). The low t1/2 estimate of 0.64 hr was reflective of the early loss of analytical sensitivity to detect Risedronate prior to reaching a log-linear terminal elimination phase. Due to the poor bioavailability of Risedronate, almost all of the dose was recovered in feces, with only 0.8% present in urine. After oral doses ranging from recovery of the dose was also largely in feces, but lower and more variable , with present in urine. the reason for the incomplete and variable recovery was attributed to errors in quantitative sample collection of feces and radioanalysis of carcass.

A subsequent single dose study was performed in dogs given unlabeled Risedronate over a dose range of ,, with drug levels in serum determined by the . As indicated by the short apparent Tmax) observed over the dose range, absorption was rapid. After reaching Cmax, serum drug levels rapidly decreased in a multi-exponential manner, with elimination half-life estimates ranging from
The estimate of 2.54 hr for the 0.2 mg/kg dose resulted from a loss of analytical sensitivity. A comparison of dose-normalized AUC(0-24 hr) and Cmax as a function of dose indicated the kinetics were dose-dependent over the dose range, becoming nonlinear at doses greater than or equal to 4 mg/kg.

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Repeated- Dose Pharmacokinetics Studies

Study	Species	Route	Duration:	Title:	Doses (mg/ kg/ day)
G28	Rat	oral	28 d (daily)	A Toxicokinetic Study in Male Rats Following once Daily Oral Administration of Risedronate for 28 Consecutive Days	2, 4, 8, 16, 32, 64
G29A/ B	Rat	oral	13 wk (daily)	A 13-Week Oral Toxicity Study of NE-58095 in the Albino Rat Followed by a 6-Week Recovery	4, 8, 16, 32, 64
G30A/ B	Rat	oral	26 wk (daily)	A 26-Week Oral Toxicity Study of NE-58095 in the Albino Rat	4, 8, 16, 32
G31A/ B	Dog	oral	13 wk (daily)	A Subchronic Study to Evaluate Hepatotoxicity in the Dog When Administered via Capsule	6, 8, 12
G32A/ B	Dog	oral	52 wk (daily)	52-Week Repeated Dose Chronic Oral Toxicity Study of NE-58095 Administered Via Capsule to Dogs	4, 8, 16, 32

Repeated dose pharmacokinetics of Risedronate have been evaluated in several rat and dog toxicity studies at once-daily doses ranging from 2 mg/kg to 64 mg/kg for 28 days to 52 weeks time. Animals were fasted from 4 hr pre-dosing to 2 hr post-dosing. In these studies, time to reach steady-state was evaluated by comparing AUC0-24 hr as a function of sample day. Dose proportionality was tested by comparing dose-normalized AUC0-24 hr as a function of dose at steady-state, or the last sample date if steady-state was not reached. Differences in the kinetics due to sex were also evaluated. AUC and Cmax data from these studies at steady state, or the last sample day if steady-state was not reached, are tabulated below. For rats, steady-state was generally reached by at least Day 14, for the lower doses of 2, 4, and 8 mg/kg. Accumulation ratios were low for doses of 2-8 mg/kg, becoming progressively larger as dose increased to 64 mg/kg. The reason for this accumulation at higher doses appears to be due to enhanced absorption. The dose-response relationship for systemic drug accumulation suggests that accumulation will be negligible at the clinical dose. Dose proportionality at steady-state was seen up to 8 mg/kg for the 13- and 26-week studies with the kinetics becoming nonlinear above this dose. In the 13-week study conducted in dogs steady-state did not appear to be reached by Day 90 at the highest dose of 12 mg/kg/day (Table 7), but accumulation ratios, based on serum AUC0-24 hr , were low for all doses. Progressive increases in dose-normalized AUC0-24 hr with dose at steady-state indicated dose-dependent kinetics over the dose range examined. For the 52-week dog study, steady-state was reached early for the 4 and 8 mg/kg doses; animals in the higher dose groups (16 and 32 mg/kg/day) died prior to Day 182, precluding any analysis for attainment of steady-state. No systemic accumulation of Risedronate was seen in this study for the 4 and 8 mg/kg doses.

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Summary of Toxicokinetic Data (AUC and C max) from Animal Toxicology Studies.

	4 Week Study - Rat- G28		13 Week Study - Rat G29		26 Week Study - Rat G30		13 Week Study - Dog G31		52 Week Study - Dog G32	
Dose (mg/ kg)	AUC (0- 24) (ng* hr/ mL)	C max (ng/ mL)	AUC (0- 24) (ng* hr/ mL)	C max (ng/ mL)	AUC (0-24) (ng* hr/ mL)	C max (ng/ mL)	AUC (0- 24) (ng* hr/ mL)	C max (ng/ mL)	AUC (0- 24) (ng* hr/ mL)	C max (ng/ mL)
2	23.7 (25)	10.1 (49)	–	–	–	–	–	–	–	–
4	34.0 (17)	16.0 (48)	152 (53)	84.7 (72.2)	78 (70)	19.5 (105)	–	–	624 (146)	196 (63)
6	–	–	–	–	–	–	620 (55)	295 (69)	–	–
8	81.9 (47)	43.6 (32)	285 (135)	110 (123)	275 (96)	86 (113)	1377 (66)	416 (66)	1893 (86)	483 (56)
12	–	–	–	–	–	–	6532 (103)	1196 (34)	–	–
16	299 (65)	118 (72)	2537 (55)	1295 (69)	1267 (119)	372 (150)	–	–	NS	NS
32	2306 (130)	910 (148)	26700 (103)	4332 (43)	8454 (85)	1902 (102)	–	–	NS	NS
64	12900 (73)	3996 (77)	49500 (75)	8892 (128)	–	–	–	–	–	–

Values are mean (% coefficient of variation)
– = Dose level not used in this study; NS = no sample due to unscheduled deaths
AUC and C max data obtained at steady- state or the last sample day if steady- state was not achieved

Animal and human pharmacokinetic data were compared in terms of multiples of human exposure to provide an index of human safety. Multiples of human exposure were calculated from AUC obtained on the terminal sampling day in toxicity studies, expressed as a ratio to the steady-state AUC from pharmacokinetics studies in humans. Human data were from normal male volunteers receiving a 30 mg oral dose. Clinical AUCss was estimated as the product of the single dose AUC(0-24 hr) and the median urinary excretion accumulation factor (2.77) that was observed following oral administration of 30 mg for 13 days. The reported value (40.9 ng*hr/mL) is the geometric mean. For rats, relative exposure based on AUC progressively increased with dose from and tended to increase as the duration of dosing increased past 4 weeks. At the NOAEL in rats (8 mg/kg), relative exposure ranged from For dogs, AUC-based relative exposure also progressively increased with dose from about Relative exposure at the minimally toxic dose in dogs (8 mg/kg) ranged from , and at the NOAEL in dogs), relative exposure was approximately 15, indicating a reasonable safety margin. However, on a mg/m^2 basis, for dogs this dose is 8-times the human dose, and for rats it is only 2.6-times the human dose. In addition, at 8 mg/kg and at higher dose levels the exposure levels (AUC) in rats (>13 wk) and dogs are non-linear.

AUC in Animals Expressed as Multiples of Corresponding Human AUC

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Dose (mg/ kg)	4 Wk Study - Rat G28	13 Wk Study - Rat G29	26 Wk Study - Rat G30	13 Wk Study - Dog G31	52 Wk Study - Dog G32
2	0.58	–	–	–	–
4	0.83	3.72	1.91	–	15.3
6	–	–	–	15.2	–
8	2.00	7.0	6.7	33.7	46
12	–	–	–	160	–
16	7.3	62	31.0	–	NS
32	56	653	207	–	NS
64	315	1210	–	–	–

– = Dose level not used in this study; NS = no sample due to unscheduled deaths
Multiples of human exposure were calculated from AUC obtained on the terminal sampling day in nonclinical toxicity studies, expressed as a ratio to the estimated steady- state AUC from pharmacokinetics studies in humans receiving a 30 mg oral dose (40.9 ng* hr/ mL).

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SUMMARY Of PK and ADME Studies

In summary, oral Risedronate is poorly but rapidly absorbed from the stomach (T max ~1h). It is highly protein bound and is primarily absorbed by bone. Some distribution is seen in the kidney because this is the route of elimination. IV doses are not eliminated in the feces or found in the bile confirming that the kidney is the only route of elimination. Distribution to bone and elimination via urine result in a half life of about depending on species and dose. No metabolites were detected in blood, feces or (cannulated) urine however in normally collected urine some degraded products were found. Steady-state exposure is reached within 14 days at doses below 8 mg/kg/day (15-fold greater than the recommended clinical dose) with little or no systemic accumulation. At higher doses systemic accumulation is seen over time, and is more significant in rats than dogs. Where possible, analysis of dose proportionality at steady-state indicates linear kinetics at doses up to 8 mg/kg/day for rats, becoming nonlinear at higher doses and also nonlinear at 8 mg/kg in rats and dogs in studies longer than 13 weeks. No sex differences in exposure are seen in rats and dogs after single and multiple doses.

Note added in proof:

The sponsor has FAXed a statement that an amendment is being submitted which significantly changes the plasma protein binding. This study has not been received or reviewed yet. The new values are approximately 98% for rats, 35% for dogs and 25% for humans.

Reviewers Overall Summary and Conclusions:

Summary of Preclinical Toxicity Studies:

Bone quality:

In Paget's disease, the primary abnormality is a local increase in osteoclast activity that results in increased bone resorption and bone turnover with formation of structurally fragile osseous tissue at specific sites or lesions. Current therapies are bisphosphonates and Calcitonin. There are no animal models of Paget's disease. The following pharmacology data from animal studies support the use of Risedronate in the treatment of Paget's disease:

In several models of osteoclast-mediated bone resorption and bone loss Risedronate has a dose-dependent inhibitory effect on bone resorption and a concomitant suppression of bone turnover.
In tumor models, Risedronate reduced hypercalcemia and osteolysis, primarily through an anti-osteoclastic action. There were also some beneficial effects in models of arthritis. Effectiveness was observed upon daily or intermittent dosing, in prevention and treatment paradigms, and was sustained for a considerable amount of time after discontinuation of dosing.

Risedronate did not appear to have deleterious effects on mineralization, spontaneous fracture incidence, or mechanical bone strength.

Toxicity Studies:

Single oral doses (~2 g/kg) were tolerated by rats, mice and rabbits (dogs vomited doses greater than 50 mg/kg). On a mg/m^2 basis this is 666 (for rats) and 333 (for mice) times the proposed human exposure (0.5 mg/kg). Higher doses were lethal. Rabbits appeared to be more sensitive to Risedronate but were not extensively tested.

Single IV doses, (3 mg/kg in rabbits, 4 mg/kg in dogs, and 25 mg/kg in mice and rats) were well tolerated. Higher doses were lethal. These IV doses are about 1% of the maximum tolerated oral doses. This is predicted due to an expected absorption of only about 1% of the oral dose.

Liver and kidneys were noted as possible target organs in many of the acute studies. In addition, drug related gastric effects were noted in dead dogs, rats and rabbits given drug IV as well as orally. Gastric edema was also noted in the 2-day IV study in rats. It is interesting to note that evidence of gastric irritation was noted not only in oral studies but also in IV studies even though there is no direct contact of drug with the luminal side of the digestive system (ADME studies show no evidence of biliary secretion) after IV exposure.

The NOAELs for repeated dose oral Risedronate in dogs, rats and mice are summarized in the following table.

Oral Toxicity Studies:				Fold-recommended human dose:		
Species:	**NOAEL (mg/kg/d)**	**NOAEL (mg/m^2)**	**Principal Target Organs:**	**based on mg/kg**	**based on mg/m^2**	**based on AUC**
Dog < 6-month	4	80	stomach, liver, kidney, testes	8 X	4.3 X	
Dog 1 year	4	80	stomach, liver, kidney, testes	8 X	4.3 X	15.3 X
Dog 2 year	>2	>40	doses too low	>4 X	> 2.1 X	
Rat < 6-month	8	48	stomach, liver, kidney	16 X	2.6 X	
Rat 6 month	8	48	stomach, liver, kidney	16 X	2.6 X	7 X
Rat 1-year	> 4	>24	doses too low	>8 X	>1.3 X	
Mouse < 6 mo.	8	24	stomach, liver	16 X	1.3 X	

Recommended human dose = **30 mg/60 kg = 0.5 mg/kg = 18.5 mg/m^2**.

The toxicities seen in all species were hepatic toxicity (increased AST, ALT...lesions and atrophy) and gastric irritation and gastric distress leading to poor food consumption. In dogs and rats moderate testicular toxicity including spermatid arrest and tubular degeneration, severely increased neutrophils, mild renal tubular nephropathy and necrosis were evident. Erosion of the GI tract from the esophagus to the large intestine and inflammation of lungs, acinar pancreas, and lymph nodes was also observed in dogs.

Risedronate appears similar to other bisphosphonates in its potential to irritate the GI system directly. While this effect of Risedronate does not seem to be more severe or less severe than other bisphosphonates at this time, the question has not been conclusively answered by these investigations. The mechanism(s) of this effect remains unknown.

Risedronate also caused slight ocular and skin irritation but was not antigenic in guinea pigs.

Reproductive toxicity:

In the rat, treatment with Risedronate reduced fertility by inhibiting ovulation and implantation at 16 mg/kg/day (equivalent to 5.5 times the 30-mg human dose on the basis of surface area comparison, mg/m^2). It is unclear from the data whether there was a reduction in male rat fertility at doses of 40 mg/kg/day or higher. When dams were treated with 16 or 80 mg/kg/day there was a reduction in viability of neonates. However, at these doses (16 and 80 mg/kg/day) maternal toxicity, ie, reduced food consumption and body weight gain, was also observed. At maternal doses of , the incidence of incompletely ossified or unossified fetal sternebrae or skull was decreased. At doses of 16 mg/kg/day and higher the incidence of incompletely ossified or unossified sternebrae or skull was increased. Cleft palate was observed in fetuses from dams treated with 3.2 and 7.1 mg/kg/day. At doses of 3.2 mg/kg/day and higher there was periparturient mortality possibly related to hypocalcemia occurring around parturition time. This effect was also seen when treatment was discontinued before parturition. When dams were treated throughout parturition, there was an increased number of stillborn fetuses at 8 mg/kg/day. Rabbits treated with 50 mg/kg/day all died, probably due to esophageal ulceration and resulting systemic toxicity. Abortion and premature delivery occurred at 10 mg/kg/day. No fetal malformations were noted at doses up to 10 mg/kg/day

Genetic toxicity / Carcinogenicity:

Risedronate did not show any evidence for genetic toxicity.

- The Rat Micronucleus Assay was negative for chromosomal aberrations.
- The Ames Assay does not indicate a mutagenic potential for Risedronate +/- S-9.
- The E. coli. Reverse Mutation Assay does not indicate a mutagenic potential for Risedronate +/- S-9.
- The CHO-HGPRT Assay indicates that Risedronate does not induce mutations in the presence or absence of metabolic activation with S-9.
- Risedronate did not induce unscheduled DNA synthesis in cultured rat hepatocytes.
- Risedronate was not clastogenic in CHO cells +/- S-9.
- Rat and mouse 2-year carcinogenicity studies are under way. NDA will be reviewed prior to finding the outcome of the studies. Results will be reported in a sNDA for the osteoporosis indication.

Metabolism Studies:

Oral Risedronate is poorly but rapidly absorbed from the stomach (T max ~1h). It is highly protein bound and is primarily absorbed by bone. Some distribution is seen in the kidney because this is the route of elimination. IV doses are not eliminated in the feces or found in the bile confirming that the kidney is the only route of elimination. Distribution to bone and elimination via urine result in a half life of about hours depending on species and dose. No metabolites were detected in blood, feces or (cannulated) urine however in normally collected urine some degraded products were found. Steady-state exposure is reached within 14 days at doses below 8 mg/kg/day with little or no systemic accumulation. This dose is 15-times the human dose (0.5 mg/kg) but on a mg/m^2 basis, for dogs it is 8-times the human dose, and for rats it is only 2.6-times the human dose. At higher doses a great deal of systemic accumulation is seen over time, being more significant in rats than dogs. Analysis of dose proportionality at steady-state indicates linear kinetics at doses below 8 mg/kg/day for rats, becoming nonlinear at higher doses and also nonlinear at 8 mg/kg in rats and dogs in studies longer than 13 weeks. No sex differences in exposure are seen in rats and dogs after single and multiple doses.

Pharmacology Recommendation:

Pharmacology recommends approval of Actonel (Risedronate) for the treatment of Paget's Disease of Bone provided appropriate caution is expressed in the label regarding potential toxicity.

/S/

Gemma Kuijpers, Ph.D.
Pharmacologist

/S/

Daniel T. Coleman, Ph.D.
Pharmacologist.

March 26, 1996

Sponsor: Proctor and Gamble Pharmaceuticals, Inc.; Norwich, New York 13815

Date Submitted: 8/9/94 **Date Received:** 8/10/94

REVIEW OF REPRODUCTIVE TOXICOLOGY AMENDMENT

DRUG: Risedronate Sodium; NE-58095

CATEGORY: Inhibitor of Bone Resorption

PROPOSED CLINICAL INDICATIONS: Prophylaxis and treatment of post-menopausal osteoporosis; treatment of osteoporoses of other etiologies, including bone loss secondary to osteoarthritis; treatment of hypercalcemia of metastatic disease.

CLINICAL STATUS: Phase III 3-year fracture analyses studies are scheduled for completion in February, 1997.

REVIEW OF PROJECT No. 995.09.00-AA
General Fertility and Reproductive Performance Study Using NE-58095 in Rats

NOTE: The in-life portion of this study was done 4/91 through 7/91. The final report was dated 9/93. Lot: 12798-004B Distribution 09E. The testing facility was the sponsor's.

PURPOSE: as stated by the sponsor in the Summary, the objective of this study was an assessment of the effects of risedronate sodium on gonadal function, mating behavior, conception rate and early and late stages of pregnancy in male and female rats treated orally by gavage.

EXPERIMENTAL DESIGN: C.f. Appendix I. Note that the time frame of drug exposure is consistent with that traditionally referred to as "Segment I"; while the sponsor does not adhere to this notation, for the purposes of this review Project 995.09.00-AA is called the Segment I study.

The experiments were conducted using male breeder and virgin female Sprague Dawley rats . Both male and females were treated in this study. Doses were administered once daily by gavage, as follows: 0 (vehicle control), 2.5, 10, 40, or 160/80; to treatment groups initially comprised of 25 rats/sex. All animals were fasted four hours before and two hours following drug administration, for a total of six hours in every 24 hour period.

Males received drug for at least 60 days prior to mating. Due to excessive mortality in the high-dose treatment group, the initial 160 mg/kg/day dose was reduced to 80 mg/kg/day on treatment Day 16. Body weights and food consumption in males were measured weekly during the course of risedronate treatment prior to mating. Dosing was terminated at conception.

Females received drug for at least 14 days prior to mating, and high-dose treatments

were reduced from 160- to 80 mg/kg/day on treatment day 5. Dosing was terminated on gestational day 7. Body weights and food consumption in females were determined on days 7 and 14 of pregnancy.

During the mating period, each female was housed 1:1 with a male. Matings were between males and female within a given treatment group: i.e., control males were mated to control females, 2.5 mg/kg/day males were mated to 2.5 mg/kg/day females, etc. Estrous cyclicity of the female was evaluated daily by vaginal smears, ending with confirmation of mating by the presence of sperm or a vaginal plug. This was considered to be day 0 of gestation. Alternatively, females were considered mated if vaginal cytology and weight gain were consistent with pregnancy.

Following determination of mating, females were housed an additional 24 hrs with the male of the mating pair; then housed singly beginning on day 1 of gestation for the duration of the pregnancy. Males were sacrificed for terminal necroscopic evaluation after mating was completed. Representative samples of testis and epididymis were obtained from each male; histopathology was done on all samples from control, 40 mg/kg/day and 80 mg/kg/day animals. Additionally, organs from each animal in each treatment group were evaluated macroscopically for lesions.

Females were euthanized on day 20 of gestation, and pups were delivered by laparotomy at this time. Necropsies were done on all dams. Observations on the dam included gravid uterine weights; and the numbers of corpora lutea and resorptions. The sex, weight and viability of each pup were also noted.

All findings indicated by the Sponsor to be statistically significant were reported at the $p \leq 0.05$ level.

RESULTS

Mortality. The numbers of animals found dead or sacrificed moribund are reported in Table 1. The numbers which follow parenthetically indicate deaths attributed to gavage trauma. One female in the control group was sacrificed after early delivery of pups; this animal was not included in Table 1.

TABLE 1
Mortality Observed During the Course of the Study

	Vehicle Control	2.5 mg/kg/day	10 mg/kg/day	40 mg/kg/day	160/80 mg/kg/day
♂ drug-related	0 (0)	1 (1) 0	2 (2) 0	2 (1) 1	8 (1) 7
♀	1 (1) 0	3 (3) 0	2 (1) 1	6 (3) 3	4 (1) 3

Clinical Signs. The incidences of the following clinical signs appear dose-related: Dried sebaceous material around eyes and nares; bloody discharge around nares and penis;

presence of blood in the urine; dyspnea; sores; alopecia; malocclusion; reduced activity; weakness; dehydration; agression; struggling with dosing or difficult to dose.

Other adverse effects were apparently not dose-related.

Body Weight Gain. Body weight gains were comparable for male animals in all but the high-dose group throughout the course of treatment; thus, it is not possible to assess whether the response was dose-related. The weight gains in the 160/80 mg/kg/day animals were consistently less than those observed in all other animals, and the percent by which they differed increased monotonically as a function of treatment duration. Thus, while the mean body weight in the high-dose males was 8% less than that in control males at day 7, the decrement had increased to ~ 15% by the 60th day of treatment. These findings were corroborated by calculations of period and cumulative weight gains.

Body weight gain in females was comparable in all but the high-dose treatment groups at gestational day 7 determination; again, it was not possible to asses whether the response was dose-related. The weight gain in the high-dose females was 7% less than that observed in the controls. Coincidentally, treatment was terminated on day 7. There were no significant differences among treatment groups in body weight gain at the next determination, day 14 of gestation. These findings were corroborated by calculations of period and cumulative weight gains.

Food consumption. The findings on absolute food consumption parallel those for absolute body weight gain in both males and females. Food consumption in males was comparable in all but the high-dose treatment groups; thus, it is not possible to assess whether the response was dose-related. Among the high-dose males, consumption was significantly reduced on days 7, 56 and 60 by 19%, 16% and 21%, respectively.

Food consumption in females was comparable in all but the high-dose treatment group; again, it was not possible to assess whether the response was dose-related. The consumption of the high-dose treatment group was reduced on gestational day 7, the final day of risedronate treatment. This decline relative to the vehicle control group was 30%; in absolute terms, the high-dose group consumed ~11 g daily, in contrast to the ~16 g daily observed in the vehicle control group. On day 14, the next day for which food consumption was reported, there were no significant differences among any groups.

Ophthalmology. No data.

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Hematology. No data.

Organ weights. Neither absolute nor relative weights were reported for testes, seminal vesicles, epididymides or prostates in males.

Absolute weights of gravid uteri were reported in females; they did not differ significantly among control and treatment populations. The mean weight for all groups was 80.24 ± 3.71 g. Weights of ovaries were not reported.

Gross Pathology in males. Gross pathologic findings for unscheduled deaths were generally reported to be without abnormality , except when attributed to gavage. As previously indicated, 13 males were found dead or sacrificed moribund; of these, five deaths were attributed to gavage error. The remaining eight deaths were unaccounted for, although the

high-dose incidence of "non-gavage" mortality suggests that they were drug-related.

Other findings on necroscopic evaluation were not related to fertility or reproductive performance.

Gross Pathology in females. Gross pathologic findings for unscheduled deaths were frequently correlated with gavage trauma. As previously indicated, 16 females were found dead or sacrificed moribund; of these, nine deaths were attributed to gavage error. Two high-dose animals were reported to have moderate red glandular discoloration or fluid accumulation in the stomach. The remaining 5 deaths were unaccounted for.

There were no other significant findings on necroscopic evaluation.

Histopathology in males. Histopathology in males was done on tissue from testes and epididymides for control, 40 mg/kg/day and 160/80 mg/kg/day. Most findings occurred in the high-dose treatment group. Note that there were no data reported on sperm viability/motility nor maturation; nor was there any analysis of sperm morphology.

Testis atrophy was a common finding, although all but one report occurred at the high dose; thus, it is not possible to determine whether incidence or severity were dose-related. At 160/80 mg/kg/day there were 4 observations classified as mild, 1 as moderate and 8 as severe . Additionally, there was one report of marked atrophy at 40 mg/kg/day.

Epididymides. Epididymal atrophy of varying severity was reported in 3 high-dose males and 1 40 mg/kg/day male. From these data it was difficult to discern a dose-response relationship. Similarly, oligospermia of varying severity was observed in 7 high-dose and 1 40mg/kg/day males. 5 High-dose males were reported to have sperm granulomata. Three high-dose animals had chronic periductular inflammation.

Note that there were no data reported of actual epididymal sperm counts, sperm viability/motility nor maturation; nor was there any analysis of sperm morphology.

Histopathology in females. No data.

Effects of Risedronate Treatment on Mating, Fertility and Offspring. There are no data reported on the number of cohabitation periods/estrous cycles needed before the observation of vaginal plugs or sperm in vaginal cytology.

Copulation and Fertility Indices were calculated. There were no significant differences among treatment groups in Copulation Indices, all of which were 100%. Conversely, there was a trend toward reduction in the Fertility Index with increasing dose, which was statistically significant at 160/80 mg/kg/day: only 5 high-dose treatment females were confirmed pregnant at laparotomy on Day 20[1]. These results are reported in Table 2.

[1]One of these was omitted from subsequent statistical analyses due to experimental error.

TABLE 2
Effects of Risedronate on Fertility Index

Control	2.5 mg/kg/day	10 mg/kg/day	40 mg/kg/day	160/80 mg/kg/day
96%	91%	88%	82%	24% 5/21

The mean numbers of viable fetuses, dead fetuses and resorptions per litter, as well as the mean numbers of corpora lutea per dam, were not significantly different among control and treated females. However, the Sponsor's Tabulated Study Synopsis (Vol 1, pg iii) indicates that results for numbers of corpora lutea and resorptions in high-dose dams were reported only for the five pregnant animals. Thus, the actual consequences of risedronate exposure for these fertility parameters are not clear.

Statistically significant reductions in mean fetal weights were reported for two drug treatment groups: the 2.5 mg/kg/day treatment group (3.56g compared to 3.69g in pups born to control dams); and the 160/80 mg/kg/day treatment group (3.31g).

REVIEW OF PROJECT No. 995.09.00-AB
Study of Fertility and General Reproductive Performance Using NE-58095 in Rats - Segment I

NOTE: The in-life portion of these studies was conducted 7/91 through 3/92. The final report was dated 8/93. Lot: 12641-041A.

PURPOSE: as stated by the sponsor in the Summary, the objective of this study was an assessment of the effects of risedronate sodium on "gonadal function, estrous cycle, mating behavior, conception rate, the early and late stages of gestation, parturition and lactation, offspring development (physical and neuro) [sic] as well as the entire reproductive process."

EXPERIMENTAL DESIGN: C.f. Appendix II. Note that the total time frame of drug exposure and progeny observation spans three generations. While the sponsor has labelled this as a Segment I study, for the purposes of this review Project 995.09.00-AB is called the 3-generation study.

F_0. The experiments were conducted using male breeder and virgin female Sprague Dawley rats . Both male and females were treated in this study. Doses were administered once daily by gavage, to treatment groups initially comprised of 35 rats/sex (exception: high-dose females = 32). Doses are listed in Table 3.

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TABLE 3
Classification of Risedronate Treatment Groups in 3 Generation Study

	Control (C)	Low-Dose(LD)	Mid-Dose(MD)	High-Dose(HD)
♂	0	3.2	16	80/40
♀	0	3.2	7.1	16

Males received drug for at least 80 days prior to mating. Due to excessive mortality in the high-dose treatment group, the initial 80 mg/kg/day dose was reduced to 40 mg/kg/day on treatment Day 36. All males were fasted four hours before and two hours following drug administration, for a total of six hours in every 24 hour period, from treatment days 1-70. Thereafter males were not fasted, although dosing was continued. Body weights and food consumption were measured weekly. Dosing was terminated at conception, and males were sacrificed for necroscopic evaluation. Representative samples of testis, epididymis, prostate and seminal vesicle were obtained from each animal. Testes will be analyzed for possible histopathology, and submitted in a later amendment.

Females received drug for at least 15 days prior to mating. All animals were fasted four hours before and two hours following drug administration, for a total of six hours in every 24 hour period[2]. Dosing was terminated upon weaning of neonates (day 21 of lactation); or on gestational day 20, at sacrifice. Body weights in females were determined at weekly intervals. Total weekly food consumption was reported.

During the mating period, each female was housed 1:1 with a male. Matings were between males and females within a given treatment group. Estrous cyclicity of the female was evaluated by daily vaginal smear until the presence of vaginal plug and/or sperm in vaginal smear. This was considered to be day 0 of gestation. Each female was then returned to an individual cage.

Twenty females were randomly selected from each dose group for euthanization and laparotomy on gestational day 20. Body weights with and without the gravid uterus were recorded. The uterus of each female was excised, weighed and examined for corpora lutea, implantation sites and resorptions, as well as the numbers of viable and nonviable fetuses. The ovaries, uterus and vagina from each female were preserved. Fetuses were identified, examined externally, tagged, sexed and weighed. The left pelvic limb (femur with articulating surfaces proximal to metatarsals) was removed from one randomly selected fetus per litter and preserved for possible future histopathology. ~ One half of each litter was prepared and stained for skeletal anomalies according to the Alizarin Red S method; remaining littermates were prepared and examined for soft tissue anomalies as per Wilson's Method.

Females from each treatment group not scheduled for day 20 euthanization were transferred to nesting boxes on gestational day 18 and allowed to deliver. The day of parturition was designated lactation day 0. Litters were sexed and examined as soon as possible after delivery. Pups were weighed on lactation days 0, 4, 7, 14 and 21, and checked

[2] Females were not, however, fasted during the mating period; or on gestational day 20, if they were scheduled for sacrifice.

daily for deaths. Pups found dead prior to lactation day 4 were eviscerated and preserved for later skeletal evaluation. Photographs of malformations were archived with study records.

All lactating dams were euthanized on lactational day 2.

F_1. Four male and 4 female pups were chosen from each litter (whenever possible) for developmental evaluation and behavioral testing on lactation day 4.[3] Tests of physical development, sensory-motor coordination and reflex development were performed at appropriate ages. At weaning (lactational day 21), one/sex/litter from the F_1 generation was retained as a parent for the F_2 generation. F_1 offspring were observed at least once daily. Body weights were recorded weekly. When all F_1 animals were at least 13 weeks of age, females were mated with males from different litters within the same dose groups.

Positive identification of vaginal sperm and/or plug was considered as Day 0 of gestation. At the end of the mating period, the males were sacrificed.

The mated F_1 females were weighed on Days 0, 6, 13 and 20 of gestation. A cesarean section was performed on each female on Day 20 of gestation. The females were euthanized and the actual and corrected body weights (subtracted weight of the gravid uterus) were recorded. The uterus of each female was excised and weighed, and examined for early or late resorptions and the number of viable and non-viable fetuses. Total number of corpora lutea was determined for each ovary. Fetuses were examined, weighed and sexed.

All findings indicated by the Sponsor to be statistically significant were reported at the $p \leq 0.05$ level.

RESULTS

Mortality. A total of 30 F_0 animals were found dead or sacrificed moribund during the course of the study, as reported in Table 4. Only one of these deaths was not attributed to risedronate toxicity.[4] All females were dams scheduled for vaginal delivery; these died between gestational days 21-23, generally prior to parturition. The mortality rate in females was not dose-related. Due to the peri-parturient nature of drug-induced mortality in females, data for dams are presented in Table 4 as the ratio of the number of deaths to the number of gravid females scheduled for delivery within a given treatment group.

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[3] Failing this, litter size was standardized such that N=8 (whenever possible).

[4] One high-dose male, found dead on treatment day 5, showed signs of inflammatory and degenerative heart changes which were not attributed to risedronate.

TABLE 4
Treatment-related Mortality

Dose (ppm) ♂ ♀	Control	Low-Dose	Mid-Dose	High-Dose
♂	0	0	0	13/35
♀	0	6/13	6/13	5/8

Clinical Signs. The incidences of the following clinical signs observed in males appear dose-related: broken, worn or missing incisors; chromodacryorrhea; rales; ventral cervical edema; conjunctivitis; flaccid body tone; abnormal or elevated gait; dried, brown sebaceous material around eyes or nares; presence of blood in the urine; blood or urine staining of urogenital region; dyspnea; sores; alopecia; reduced activity; weakness; ptosis; and post-dose tremors and salivation.

There were no clinical signs observed in any female rats prior to conception. The following signs, evident from day 21 of gestation in some treated dams scheduled for normal vaginal delivery, were considered drug-related and were inevitably followed by the death of the animal: chromodacryorrhea; abnormal gait and stance; reduced activity; ptosis; tremors; piloerection; cold to touch; and dyspnea.

Body Weight Gains. Body weights in males were comparable in control and low-dose treatment groups throughout the study. Mid-dose animals began to lag behind control animals on day 64, and continued to show lower body weights through the end of the study; however, these values were consistently < 10% below control weights. The amounts by which high-dose animals lagged behind controls increased monotonically as a function of treatment duration, ranging

There were no differences in body weights among control or treated females during the entire treatment period.

Food Consumption. Food consumption in males was comparable in control and low-dose treatment groups throughout the study. Mid-dose animals began to lag behind control animals on day 50, and continued to show reduced intake through day 71; these values ranged from below control consumptions. Reductions in food consumption in high-dose animals increased monotonically as a function of treatment duration, ranging

Food consumption was reduced by 11% in high-dose females during gestational days 7-13; this finding was statistically significant. Food consumption in this group on gestational days was ~10% lower than that observed in controls; while not statistically signficant, the Sponsor considers this finding to have biologic significance. There were no other differences in

[5] Note that pre- and post-dose fasting of males was stopped at treatment day 70. Since the sponsor estimates that <1% of an oral dose is absorbed in the presence of food, there was considerably less additional body burden after day 70.

food consumption among control or treated females during pre-conceptional or gestational treatment periods. Food consumption was not measured during lactation.

Ophthalmalogy. Not done.

Hematology. Not done.

Organ Weights. The sponsor reported changes in relative male reproductive organ weights, some of which were statistically significant. I have transposed these values to percent changes relative to controls, since the original data are small numbers, and difficult to appreciate. Transposed values exceeding a 5% change are reported in Table 5. Asterisks indicate statistical significance when compared with controls.

TABLE 5
Changes in Relative Reproductive Organ Weights

Dose	Testes	Epididymides	Prostate	Seminal Vesicles
Control				
3.2	-	-	-	-
16	-	-	+17.5*	+14*
80/40	+31.6*	+14.77*	+17.5	+14*

Organ weights were not analyzed for females in this study, with the exception of the weight of the gravid uterus; these did not differ among control and treatment females.

Gross Pathology. Not done.

Histopathology. Not presently available. (The sponsor has indicated that histopathology of testes and epididymides is to be incorporated into a future amendment.)

Mating, Fertility and Reproductive Outcome. There were no data for Copulation Indices. Fertility indices, as calculated by this reviewer, are presented in Table 6. (The value in parentheses indicates the number of females in the treatment group.)

TABLE 6
Fertility Indices for F_0 Treatment groups

	Control	3.2 mg/kg/day	7.1 mg/kg/day	16 mg/kg/day
F0	94% (35)	91% (35)	94% (35)	69% (32)

As previously indicated in the experimental design, 20 females of each treatment group were scheduled for Day 20 sacrifice and laparotomy; the remaining animals were slated for normal vaginal delivery. Table 4 clearly indicates that a large number of the latter group died

during parturition.

Since risedronate is known to elicit hypocalcemia, and because hypocalcemia can cause dystocia secondary to uterine inertia, serum Ca^{++} levels were determined in some animals in each group scheduled for Day 20 sacrifice. Serum Ca^{++} levels (in mg/dl) are recounted here in Table 7. Asterisks indicate statistical significance.

TABLE 7
Serum Ca^{++} Levels on Gestational Day 20 in Laparotomized Females

	Control	Low-dose	Mid-dose	High-Dose
Mean	11.4		8.0*	
S.D.	0.84		1.57	
Range				
N	4		8	

Average changes in serum Ca^{++} ranged from lower among drug-treated females, and these reductions did not appear to be dose-related. The sponsor postulates that this "treatment effect" observed in laparotomized females is responsible for the excessive peri-parturient mortality observed in females scheduled for vaginal delivery.

The sponsor stated that "*Based upon the number of gravid animals per dose group*", the numbers of corpora lutea and the numbers of implantations per dam were reduced in the high-dose group; and the numbers of implantations were reduced in the mid-dose group. These data are presented in Table 8 (Mean ± S.D.) Asterisks indicate statistical significance.

TABLE 8
Effects of Risedronate on Fertility

	Control	3.2 mg/kg/day	7.1 mg/kg/day	16 mg/kg/day
Total # Corpora Lutea per dam	17.0±1.81	17.2±2.61	15.9±1.76	14.1±2.35*
Total # Implantations per dam	16.2±2.39	15.5±1.84	15.1±2.04*	13.6±2.21*

There were no apparent treatment-related effects in the following findings, which are presented as means for all treatment groups: incidence of prematurity (0%); fetal sex distributions (male = 48.9% and female = 51.1%); and mean fetal body weights (4.075g).

Fetal Malformations. The numbers of drug-treated litters affected by soft tissue and skeletal anomalies were not statistically significant when compared with controls. The incidence and types of anomalies noted are as follows:

- 2/20 control (one fetus each with bilateral microphthalmia or hypoplastic, ectopic kidneys);
- 1/19 low-dose (1 fetus with cleft palate);
- 3/20 mid-dose (1 fetus in each of two litters with cleft palate; 1 fetus in a third litter with hydrocephalus); and
- 1/14 high-dose (1 fetus with missing lumbar vertebrae).

On external examination, there were two incidental abnormalities: one pup in one control litter had microphthalmia; one pup in one low-dose litter had an umbilical hernia.

Skeletal variations were generally described as increases or decreases in fetal ossification. The incidences and types of variations observed are listed below in Table 9. Asterisks indicate statistical significance.

TABLE 9
Effects of Risedronate on the Occurrence of skeletal variations

	Control		3.2 mg/kg/day		7.1 mg/kg/day		16 mg/kg/day	
	# Pups	#litters	# Pups	#litters	# Pups	#litters	#Pups	#litters
Unossified 5th Sternebra	72	18	35*	13	19*	9	35	10
Unossified 6th Sternebra	29	13	*6	6	3*	2	11	5
Incomplete 4th Sternebra Ossification	18	10	3*	2	6	5	6	6
Incomplete 5th Sternebra Ossification	65	17	82	17	108*	19	50	12

Effects of Prenatal and Neonatal Risedronate Exposure on Offspring.

Mortality. The sponsor reports the following data on litter mortalities from lactation days 0-4:

- 1/13 control litters (lactational day 2, litter sacrificed upon the death of the dam).
- 2/7 low-dose litters (lactational day 1, due to litter cannibalization; and day 1, due to non-viability)
- 1/7 mid-dose litters (lactational day 0, litter sacrificed upon the death of the dam)
- 1/3 high-dose litters (lactational day 2, due to non-viability of the litter).

Clinical Signs. Among pups subsequently noted to be dead or missing[6], a constellation of clinical signs were frequently observed. These offspring were visually small, pale, cold to the touch or less active. Other clinical signs in the F_1 generation were not considered to be treatment-related.

Body Weights. Body weights among pups born to dams in the control and low-dose groups are comparable. At higher doses, there is a trend toward increased birth weight which is both statistically and biologically significant at the high dose: pup weights are greater than those observed in controls.

Food Consumption. Not applicable.

Organ Weights. Not done.

Physical Development and Reflexes. Developmental indices of maturation that normally reflect physical growth varied with body weight among treatment groups. Thus, times to pinnae detachment and eye opening were accelerated in mid- and high-dose pups. Conversely, a biphasic trend was evident in 3 trials of rotorod performance (parameter reduction at the low-dose and increase at the high dose), although it did not attain statistical significance. There were no apparent effects of drug treatment on the swim maze.

Certain reflexes known to correlate with physical development also reflected the patterns of body weight findings among treatment groups: times to surface righting were increased ~72% in mid- and high-dose pups; and grasp-holding was increased in mid- and high-dose pups. Values for negative geotaxis, galton whistle, pupil constriction and passive avoidance parameters were comparable among all groups.

Mating, Fertility and Reproductive Outcomes. I have calculated fertility indices for the F_1 generation. They are listed below, in Table 10. The numbers in parentheses represent total sample sizes.

TABLE 10
Fertility Indices in the F_1 generation

	Control	3.2 mg/kg/day	7.1 mg/kg/day	16 mg/kg/day
F1	90% (10)	100% (5)	100% (5)	50% (2)

The Sponsor reported that there were no changes in any of the reproductive parameters examined. Note, however, that due primarily to extensive F_0 maternal mortality, and to a lesser extent F_1 neonatal mortality, the sample sizes were small[7] Thus, it is not possible to evaluate the reproductive toxicity of risedronate on the F_1 generation.

[6]Missing pups were presumed cannibalized.

[7]Furthermore, only 1 of the 2 F_1 HD dams was gravid.

REVIEW OF PROJECT No. 995.09.00-AC
TERATOLOGY STUDY USING NE-58095 in RATS--SEGMENT II

NOTE: The in-life portion of these studies was conducted 7/91 through 12/91. The final report was dated 8/93. Lot: 12640-041A.

PURPOSE: as stated by the sponsor in the Summary, the objective of this study was an assessment of the ability of risedronate sodium "to produce or alter the incidence of congenital malformations in offspring of pretreated Sprague-Dawley rats and to determine the effects on early and late stages of gestation, parturition, and lactation, as well as the entire reproductive process including gonadal function, mating behavior and conception rate in F_1 animals."

EXPERIMENTAL DESIGN: C.f. Appendix III. Since the total time frame of drug exposure is limited to gestational days 7-17 for the F_0 generation, Project 995.09.00-AB is called the Segment II study for the purposes of this review.

F_0. The experiments were conducted using male breeder and virgin female Sprague Dawley rats

During the mating period, each female was housed 1:1 with a male. Matings were between untreated males and vehicle- or drug-treated females. Estrous cyclicity of the female was evaluated by daily vaginal smear until the presence of vaginal plug and/or sperm in vaginal smear. This was considered to be day 0 of gestation. Each female was then returned to an individual cage.

Females were treated from days 7-17 of gestation. Doses were administered once daily by gavage, to treatment groups initially comprised of 36 females. Doses are listed in Table 11.

TABLE 11
Classification of Risedronate Treatment Groups in Segment II Study

Control (C)	Low-Dose(LD)	Mid-Dose(MD)	High-dose (HD)
Vehicle		16	

All females were fasted four hours before and two hours following drug administration, for a total of six hours in every 24 hour period. Body weights and food consumption in pregnant females were determined at confirmation of pregnancy, and again at ~ 3 day intervals thereafter throughout gestation; in dams scheduled for delivery, weights were likewise recorded during lactation. Total weekly food consumption was reported.

Twenty-four females were selected from each dose group for euthanization and laparotomy on gestational day 20. Body weights with and without the gravid uterus were recorded. The uterus of each pregnant female was excised, weighed and examined for corpora lutea, implantation sites and resorptions, as well as the numbers of viable and nonviable fetuses. The ovaries, uterus and vagina from each female were preserved. Fetuses were identified, examined externally, tagged, sexed and weighed. The left pelvic limb (femur with articulating surfaces proximal to metatarsals) was removed from one randomly selected fetus per litter and preserved for possible future histopathology. ~ One half of each litter was

prepared and stained for skeletal anomalies according to the Alizarin Red S method; remaining littermates were prepared and examined for soft tissue anomalies as per Wilson's Method.

Females from each treatment group not scheduled for day 20 euthanization were transferred to nesting boxes on gestational day 18 and allowed to deliver. The day of parturition was designated lactation day 0. Litters were sexed and examined as soon as possible after delivery. Pups were weighed on lactation days 0, 4, 7, 14 and 21, and checked daily for deaths. Pups found dead prior to lactation day 4 were eviscerated and preserved for later skeletal evaluation. Photographs of malformations were archived with study records.

All lactating dams were euthanized on lactational day 21.

F_1. Four male and 4 female pups were chosen at random from each litter (whenever possible) for developmental evaluation and behavioral testing on lactation day 4.[8] Tests of physical development, sensory-motor coordination and reflex development were performed at appropriate ages. At weaning (lactational day 21), one/sex/litter from the F_1 generation was retained as a parent for the F_2 generation. F_1 offspring were observed at least once daily. Body weights were recorded weekly. When all F_1 animals were at least 13 weeks of age, females were mated with males from different litters within the same dose groups.

Positive identification of vaginal sperm and/or plug was considered as Day 0 of gestation. At the end of the mating period, the males were sacrificed.

The mated F_1 females were weighed on Days 0, 6, 13 and 20 of gestation. A cesarean section was performed on each female on Day 20 of gestation. The females were euthanized and the actual and corrected body weights (subtracted weight of the gravid uterus) were recorded. The uterus of each female was excised and weighed, and examined for early or late resorptions and the number of viable and non-viable fetuses. Total number of corpora lutea was determined for each ovary. Fetuses were examined, weighed and sexed.

All findings indicated by the Sponsor to be statistically significant were reported at the $p \leq 0.05$ level.

RESULTS

Clinical signs. There were no clinical signs in pregnant females in this study that appeared to be dose-related. However, many of the clinical signs noted previously with risedronate administration were observed in this study: reduced activity, abnormal stance, elevated gait, tremors, chromodacryorrhea, lacrimation, ptosis and alopecia.

Mortality. A total of 8 F_0 animals were found dead or sacrificed moribund during the course of the study. All were drug-treated dams scheduled for vaginal delivery; and all but one died between gestational Since risedronate-induced mortality in pregnant dams is generally peri-parturient, the data are presented below in Table 12 as the ratio of the number of deaths to the number of gravid females scheduled for delivery within a given treatment group.

[8] Failing this, litter size was standardized such that N=8 (whenever possible).

[9] One HD dam died on day 7 of lactation, exhibiting the same clinical signs as those that died during parturition.

TABLE 12
Treatment-related Mortality

Control	3.2 mg/kg/day	16 mg/kg/day	80 mg/kg/day
0/11	1/11	4/12	3/7

Body weight and food consumption. The body weights among control and LD dams were not significantly different throughout the course of gestation. The weights of MD and HD dams were reduced by ~4% each on gestational day 15, a finding the sponsor reported as statistically significant. Additionally, the sponsor reported that body weight change was significantly lower in the HD group on days 6-9 relative to controls (3.9g vs 11.6g, respectively).

Statistically significant reductions in food consumption were noted by the sponsor in the MD dams during gestational days 9-15); and in the HD dams

Note that all groups exhibited ~30% reductions in consumption on days15-17.

Ophthalmology. Not done.

Hematology. Not done.

Organ Weights. Organ weights were not analyzed for females in this study, with the exception of the weight of the gravid uterus; these did not differ among control and treatment females.

Gross Pathology. Not done.

Histopathology. Not done.

Effects of Risedronate on Reproductive Outcome. Table 13 outlines the effects of risedronate on the percentage of pregnancies carried to term, expressed as the percentage of gravid females at laparotomy or day 26 relative to the total number of females successfully mated.

TABLE 13
Percentage of Pregnancies Carried to Term

	Control	3.2 mg/kg/day	16 mg/kg/day	80 mg/kg/day
F_0	94%	92%	92%	81%

The sponsor stated that "*Based upon the number of gravid animals per dose group,* there were no statistically significant differences observed in the mean number of early or late resorptions, or the number and percentage of the pre-implantation losses."

Fetal Malformations. The sponsor stated that neither soft tissue nor skeletal malformations were significantly different in control and drug-treated groups. The incidence and types of anomalies noted are as follows:

- 1/23 control litters (ectopic testis)
- 3/22 low-dose litters (5/296 fetuses: one fetus in one litter simply classed as "malformed"; two fetuses from one litter with hydrocephalus, and one fetus from this litter with atrophied testis; one fetus from each of two litters with cleft palate; one of the two fetuses with cleft palate was called a "polysomatous monster", having anophthalmia, cleft palate, 2 identifiable sets of sex organs, 2 tails + 1 remnant tail, 4 hind legs and a small low-set pinna).

Note that decomposition incompatable with examiner evaluation was noted in 1 fetus/litter/dose in the mid- and high-dose groups.

The incidences and types of skeletal variations are noted below in Table 14. Asterisks denote statistical significance.

TABLE 14
Incidences of Skeletal Variations

	Control		3.2 mg/kg/day		16 mg/kg/day		80 mg/kg/day	
	# Pups	#litters	# Pups	#litters	# Pups	#litters	#Pups	#litters
Unossified 5th Sternebra	30	15	29	15	18	9	97*	21
Unossified 6th Sternebra	16	8	12	8	10	7	86*	21
Incomplete 4th Sternebra Ossification	8	7	7	5	21*	13	25	10
Incomplete 5th Sternebra Ossification	118	21	103	22	96	20	46*	14
Incomplete Skull Ossification	12	6	1*	1	4	2	34	12

Effects of Prenatal and Neonatal Risedronate Exposure on Offspring.

Mortality. The sponsor reports the following data on litter mortalities from lactation days 0-4:

- 0/11 controls
- 0/10 low-dose
- 0/8 mid-dose
- 1/4 high-dose (litter sacrificed upon death of dam)

Additionally, the sponsor tallied the total numbers of viable neonates available for culling on day 4, and noted a statistically significant reduction in the mid-and high-dose counts. These values were 168, 140, 88 and 37, respectively, for control, low-dose, mid-dose and high-dose treatment groups.

Finally, the sponsor reported that the numbers of viable neonates/litter at delivery, as well as the numbers of pups/litter on day 4 prior to standardization, were reduced in the mid- and high-dose treatment groups when compared with controls. The data are presented below in table 15. All findings are expressed as means/litter; asterisks indicate statistical significance.

TABLE 15
Effects of Risedronate on the Size of the Offspring Population

	Control	Low-Dose	Mid-dose	High-dose
Pups delivered	15.5 ± 1.51		11.5 ± 5.4	
Pups viable	15.4 ± 1.5		11.3* ± 5.3	
Pups @ Day 4	15.3 ± 1.35		11* ± 5.5	

Clinical signs. Among control pups, the most frequent clinical findings were a lack of milk on day 0, a bruised cervical area on days 0-4 and reduced activity at various intervals. Low-dose pups were sometimes observed to be without milk, and to be small and/or pale. These findings were considered by the sponsor to be incidental. Similar findings were noted in the mid-dose group; additionally, one pup was described as having ptosis, abnormal stance and gait, and yellowish skin. Ten pups in two high-dose litters were observed with chromodacryorrhea, a finding frequently associated with risedronate administration.

Shortly after weaning and "random" selection of F_1 neonates to parent the F_2 generation, all pups from the high-dose groups were found dead or euthanized, exhibiting signs consistent with those denoting risedronate toxicity: chromodacryorrhea, elevated gait, lacrimation, ptosis, piloerection, decreased activity and body drop.

Body weights. There was an apparent biphasic response of neonatal body weight to risedronate exposure, when compared with control weights. The greatest increases were seen in the low-dose group, lesser increases in the mid-dose group and frank reductions observed in the high-dose group. These findings, seen at all time points on which body weight was recorded from lactation days 0-21, are reproduced below for day 4 (prior to culling) in Table 16. Asterisks denote statistical significance. The sponsor dismissed the increases in weight seen at the low and mid-doses as incidental, and attributed the loss at the high-dose to drug treatment.

TABLE 16
Effects of Pre- and Neonatal Risedronate Exposure on Neonatal Body Weight

	Control	3.2 mg/kg/day	16 mg/kg/day	80 mg/kg/day
Body weight	9.5 ± 1.32	10.8* ± 1.34	10.5* ± 1.62	8.9* ± 1.05

Food consumption. Not done.

Organ weights. Not done.

Ophthalmology. Not done.

Physical development and reflexes. Significant increases in latencies to pinna detachment and incisor eruption were observed in pups born to high-dose dams, by 0.3 and 8.6 days, respectively. A significant reduction in the number of errors in the 4th trial of the M-maze was observed in the low-and mid-dose groups, which the Sponsor termed incidental. No significant differences were observed among control or drug-treated animals for the following parameters: surface righting, negative geotaxis, grasp/holding, Galton whistle, eye opening, pupillary constriction, rotorod performance, or spontaneous locomotor activity.

Mating, fertility and reproductive outcomes. The fertility indices for the F_1 generation are listed below in Table 17. (The value in parentheses indicates the number of females in the treatment group.) While statistics were not reported for these results, and although sample sizes are small, there appears to be a trend toward reduced fertility in risedronate-exposed animals.

TABLE 17
Effects of Pre- and Neonatal Risedronate Exposure on Fertility in the F_1 Generation

	Control	3.2 mg/kg/day	16 mg/kg/day	80 mg/kg/day
F1	90% (10)	70% (10)	71% (7)	0[10]

All F_1 dams were scheduled for laparotomy, and none died during gestation. There were neither spontaneous abortions nor early deliveries. There were no significant differences in body weights of dams nor body weight changes. The sponsor stated that "*Based upon the number of gravid animals per dose group*", there were no significant differences among control and drug-exposed populations in mean numbers of early or late resorptions, or the numbers and percentages of pre-implantation losses.

Fetal Malformations. The sponsor stated that neither soft tissue nor skeletal malformations were significantly different in control and drug-treated groups. The incidence and types of malformations noted are as follows:

[10]Recall that there were no living progeny at the time of F_1 mating.

- 0/9 control litters
- 1/7 low-dose litters (1 fetus with a dome-shaped cranium)
- 1/5 mid-dose litters (1 fetus with gastroschisis and club foot)

The numbers of fetuses per litter and birth weights were comparable in control and treatment groups.

The sponsor concluded that this study demonstrated a maternal NOEL of 3.2 mg/kg/day ; a developmental NOEL of 16 mg/kg/day; and that, "Based upon this data, test article NE-58095 is considered not teratogenic."

REVIEW OF PROJECT No. 995.09.00-AD
TERATOLOGY STUDY USING NE-58095 in RABBITS--SEGMENT II

NOTE: The in-life portion of these studies was conducted 12/91 through 1/92. The final report was dated 8/93. Lot: 12640-041A.

PURPOSE: as stated by the sponsor in the Summary, the objective of this study was an assessment of the ability of risedronate sodium "to produce or alter the incidence of congenital malformations in offspring of pretreated rabbits".

EXPERIMENTAL DESIGN: C.f. Appendix IV. Since the total time frame of drug exposure is limited to gestational days 6-18, Project 995.09.00-AD is called the Segment II (Rabbit) study for the purposes of this review.

Experiments were conducted using virgin female New Zealand White rabbits Animals were housed individually for the duration of the study, and artificially inseminated with semen from proven bucks. Prior to insemination, human chorionic gonadotropin was administered to females via marginal ear vein. The date of insemination was designated gestational day 0.

Females were treated with vehicle or risedronate from days 6-18 of gestation. Doses were administered once daily by gavage, to treatment groups initially comprised of 18 animals. Doses are listed in Table 18.

TABLE 18

Risedronate doses (mg/kg/day) Administered to Rabbits in Segment II Study

Control (C)	Low-Dose(LD)	Mid-Dose(MD)	High-dose (HD)
Vehicle		10	

All females were fasted four hours before and two hours following drug administration, for a total of six hours in every 24 hour period. Body weights were determined at insemination, and again at ~ 3 day intervals thereafter throughout gestation. Food consumption was recorded every two days "or as appropriate".

All term females from each dose group were euthanized prior to laparotomy on gestational day 29. Body weights with and without the gravid uterus were recorded. The uterus of each pregnant female was excised, weighed and examined for corpora lutea, implantation

sites and resorptions, as well as the numbers of viable and nonviable fetuses. The ovaries, uterus and vagina from each female were preserved. Fetuses were identified, examined externally, tagged, sexed and weighed. The left pelvic limb (femur with articulating surfaces proximal to metatarsals) was removed from one randomly selected fetus per litter and preserved for possible future histopathology. ~ One half of each litter was prepared and stained for skeletal anomalies according to the Alizarin Red S method; remaining littermates were prepared and examined for soft tissue anomalies as per Wilson's Method. Photographs of malformations were archived with study records.

All findings indicated by the Sponsor to be statistically significant were reported at the $p \leq 0.05$ level.

RESULTS

Clinical signs. Clinical signs were apparent in both control and treatment groups. The occurrences of the most common are listed below in Table 19. Flaccid body tone, as well as alopecia, were previously noted with risedronate administration in rats.

TABLE 19
Clinical Signs Observed in Segment II (Rabbit) Studies

	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	50 mg/kg/day
Diarrhea	1		2	5
Minimal feces	1	1	3	8
Flaccidity	1		3	7

Mortality. Twenty six rabbits died during the course of the study. Although the Sponsor attributed all mortality to risedronate, necropsy findings for all premature decedents in the LD and MD treatment groups are consistent with misgavage. Data are summarized in Table 20.

TABLE 20
Treatment-related Mortality

	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	50 mg/kg/day
No. of Deaths	0/18	3/18	3/18	18/18

Body weight and food consumption. Body weights of high-dose does were significantly lower than controls on gestational days 12 and 15 (-9% and -12%, respectively). It is likely that this reflected significant toxicity, since 6/13 does died in these 3 days. Body weights of females in other treatment groups were not affected by risedronate.

Food consumption in high-dose does was reduced by **for most of the treatment duration.** All females in this treatment group were dead by Day 24. Food

consumption in other treatment groups was not affected.

Ophthalmology. Not done.

Hematology. Not done.

Organ Weights. Organ weights were not reported.

Effects of Risedronate on Reproductive Outcome. After artificial insemination, the total number of animals impregnated was ascertained by inference at laparotomy, whether scheduled (i.e., Day 29) or premature (due to death of the animal, spontaneous abortion, premature delivery, etc.). The percentage of pregnancies carried to term was calculated as follows:

$$\% \text{ pregnancies carried to term} = 100 * \frac{\text{number of animals pregnant at Day 29 laparotomy}}{\text{total number of animals impregnated}}$$

These data are presented in Table 21.

TABLE 21

Effects of Risedronate on % of Pregnancies Carried to Term in Rabbits

	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	50 mg/kg/day
% carried to term	100	85	71	0

Among data obtained from all treatment groups, statistically significant differences relative to controls were not observed in total numbers of corpora lutea or implantation sites. Among LD and MD groups,[11] there were no differences in pre- or post-implantation losses, viable fetuses nor early or late resorptions relative to controls; furthermore, fetal sex distrubutions and mean body weights were not altered as a result of risedronate treatment.

Fetal Malformations. The Sponsor stated that, "There were no statistically or biologically significant differences in the number of fetuses or litters exhibiting malformations or skeletal variations from the test article-treated does when compared to the control group." The incidences and types of anomalies noted were as follows:

- 3/16 control litters (3/126 fetuses: 1 dead fetus with craniorachischisis, gastrochisis, absent right rear appendage, protruding tongue, facial dysmorphia and forepaw syndactyly that was not evaluated for skeletal or visceral malformations; 1 fetus with bulbous ribs; and 1 fetus with cystic dilatation of cerebral ventricles).
- 3/11 low dose litters (4/74 fetuses: two fetuses from 2 litters with cystic dilatation of the cerebral ventricles; 1 fetus with bifurcated rib)
- 2/10 mid dose litters (2/70 fetuses: 1 with a missing rib; and one with fused sternebrae)
- **No litters from high dose females were examined for malformations**

[11] These latter observations were not made for progeny of HD females.

Seg. 1 LD LMD HMD HD ♂♀

2.5 10 40 160/80

3.2 10 40 ♂

3.2 7.1 ♀

The incidences and types of skeletal variations noted did not differ among control and drug-exposed fetuses.

Summary and Evaluation. *Segment I.* The sponsor reports that treatment of male rats with risedronate 160/80 mg/kg/day for 60 days prior to initial cohabitation, and until conception, results in increased mortality: 1/25 (4%) mid-dose- and 7/25 (28%) high-dose deaths were drug-related. Food consumption and body weight were reduced in high-dose males by ~18% and 15%, respectively, by day 60 of treatment. The sponsor also reported that a few mid-dose, and many high-dose males showed some histopathology of reproductive organs: testis atrophy, with some degree of loss of germinal epithelium, reduction in tubule size, reduction in sperm count and slowing or arrest of spermatogenesis; and epididymal atrophy, accompanied by sperm granulomata, oligospermia, periductular chronic inflammation and loss of epithelial integrity.

Treatment of female rats with risedronate 160/80 mg/kg/day for 15 days prior to initial cohabitation, through conception and terminating on day 7 of gestation results in increased treatment-related mortality at all doses ≥ 10 mg/kg/day: 1/25 (4%) at 10 mg/kg/day, 3/25 (12%) at 40 mg/kg/day and 3/25 (12%) at 160/80 mg/kg/day females were found dead or sacrificed moribund during the course of treatment. Food consumption and weight gain were reduced in high-dose females by 30% and 7%, respectively, on gestational day 7. Treatment was discontinued on this day, as per the protocol; and both parameters were not significantly different from those observed in controls by gestational day 14.

The fertility index in the high-dose treatment mating pairs was reduced to 24%, from 95% in vehicle control animals.

Weights on gestational day 20 in progeny of high-dose mating pairs were smaller than those in other treatment groups (mean=3.31g in high-dose pups vs 3.60g in controls).[12]

Three-generation study. F_0. Risedronate-related signs of systemic toxicity were seen in mid- and high-dose males, and in females.of all treatment groups. Mortality was observed in 13/32 (41%) high-dose males. All mortality in females was peri-parturient: among confirmed pregnant females scheduled for vaginal delivery, the incidences of mortality were 6/13 (42%), 6/13 (42%) and 5/8 (62%) in low- mid- and high-dose groups, respectively.

Weight gain and food consumption were slightly reduced in mid-dose males and significantly reduced in high-dose males

There were no significant changes in body weight gains among treatment groups in females, although food consumption was ~10% lower in the high-dose group during days 8-21 of gestation.

Increases were reported in relative organ weights in the high-dose treatment group when compared with controls for testes (32%), epididymides (15%), prostates (17.5%) and seminal vesicles (14%). Samples were taken for histopathologic analysis, but results have not been submitted by the Sponsor to date.

Copulation indices were not calculated, nor were data provided which would permit Division assessment. From available data, I calculated fertility indices for each group: there were no appreciable differences among control, low-dose or mid-dose dams in F_0 or F_1, for

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[12]It is unlikely that the reduction in birth weight reported for the 2.5 mg/kg/day mating pairs is biologically significant.

which the grand mean is approximately 95%. However, this value declined among high-dose F_0 females, in whom the index was only ~69%. (p. 50)

As indicated previously, all mortality in females occurred in animals scheduled for delivery and was peri-parturient. The sponsor considered the hypothesis that deaths were due to uterine inertia secondary to risedronate-induced hypocalcemia, and drew blood samples for serum Ca^{++} analysis from laparotomized females. Serum Ca^{++} levels were lower by in drug-treated animals, and the reductions were not dose-related.

Additional findings among laparotomized females included a 17% decline in the number of corpora lutea in high-dose dams, and a correspondant 16% reduction in the number of implantations. There was also a reduction in the number of viable fetuses/litter in the high-dose dams when compared with controls (13 vs 15, respectively). This trend, although not statistically significant, is of clear biological significance; furthermore, it is corroborated by neonatal mortality data calculated from dams scheduled for delivery, and presented below in Table 17 (see **"% Live Births"**).

F_1. N.B. Due to the small numbers of litters born, the validity of F_1 findings is questionable.

Among fetal malformations listed, the incidence of cleft palate in low- and mid-dose pups is suggestive (1/19 and 2/20, respectively), although no cases were observed in the high-dose group.[13] There were many skeletal variations reported, although no dose-response relationship was detected.

Among groups of F_0 dams scheduled for delivery, the incidence of F_1 litter non-viability was 0%, 29%, 0% and 33% from control, low-dose, mid-dose and high-dose treatment groups, respectively. Body weights in pups born to drug-treated dams were increased by 8-14% in the high-dose group.

Developmental indices of maturation that reflect physical growth varied directly with birth/body weight among treatment groups. These include times to pinnae detachment and eye opening, times to surface righting and duration of grasp-holding. Thus, these parameters were accelerated in high-dose pups, and to a lesser extent in mid-dose pups.

There were no reported changes in any reproductive parameters examined in the F_1 generation. However, recall that there were only 2 females in the high-dose F_1 generation, a number insufficient to evaluate.

Segment II. F_1. Prior to the onset of parturition, there were no risedronate-related signs of systemic toxicity in treated dams. The incidences of mortality in F_0 dams were 0, 9%, 33% and 43% in control, low-dose, mid-dose and high-dose groups, respectively. All mortality occurred in females scheduled for delivery; and all deaths but one were peri-parturient. (The remaining dam died on lactational day 7.)

Statistically significant reductions in weight gain were reported: 4% each in mid-dose and high-dose groups on gestational day 15; as well as a 75% reduction in body weight change on days 6-9 in the high-dose group. Additionally, statistically significant reductions in food consumption ranging from 5-17% were reported in mid- and high-dose dams over intervals spanning gestational days 9-17.

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[13]Recall that only 14/20 high dose females scheduled for laparotomy were gravid; it is likely that this sample size is too small to permit detection of a relatively rare event.

There were no ophthalmology, hematology, gross pathology or histopathology data; nor were organ weights reported.[14]

The sponsor reported that several high-dose dams that had been successfully mated were not pregnant 20-22 days thereafter. For this reason, I calculated fertility indices. While lacking statistical confirmation, there is an apparent reduction in fertility in the high-dose group relative to the grand mean for control, low-dose and mid-dose populations (81% vs ~93%, respectively); this is consistent with findings reported in the Segment I and 3-generatons studies. The sponsor noted no differences among control and drug-treated pregnant females in the following parameters: numbers of corpora lutea, numbers of pre- or post-implantation losses, numbers of early and late resorptions, incidences of soft tissue or skeletal malformations, and incidence of litter mortality.

F_1. N.B. Due to the small numbers of litters born, the validity of F_1 findings is questionable.

Statistically significant reductions were noted in the total numbers of viable neonates in the mid- and high-dose groups on lactational day 4. This reflected two independent drug-related toxicities:

- the reduced numbers of litters delivered in these groups (11, 10, 8 and 4 litters from control through high-dose, respectively); and
- a statistically significant reduction in the mean numbers of viable pups/litter on lactational days 0 (~25% in the mid- and high-dose groups) and 4 (28% and 39% in the mid- and high-dose groups, respectively).

Clinical signs consistent with risedronate toxicity were noted in pups born to mid- and high-dose dams. Shortly after weaning, all high-dose pups were found dead or euthanized.

The response of neonatal body weight to maternal risedronate exposure was biphasic. Statistically significant changes relative to controls were +11.4%, +10.5% and -6.3% for low, mid- and high-dose groups, respectively.

Neonatal food consumption, organ weights and ophthalmology were not assessed. There was only one finding of statistical and biological significance among indices of physical and reflex development: a 100% increase was observed among high-dose pups in the latency to incisor eruption While the latency to appearance of incisors frequently parallels rank order of body weight, it seems likely that the effects of risedronate on calcium homeostasis also contributed to this observation.

Fertility of the F_1 generation was reduced in low- and mid-dose animals (70% and 71%, respectively).

The numbers of fetuses per litter and birth weights were comparable in control and treatment groups. While anomalies were noted in the F_2 generation of low- and mid-dose litters, the sponsor concluded that the incidence was not statistically different from controls.

Segment II (Rabbit). The incidences of mortality in risedronate-treated does were 0%, 17%, 17% and 100% from vehicle control through HD, respectively. Given the paucity of treatment-related clinical signs, these numbers are excessive. A review of necropsy data suggest that the LD and MD decedents were victims of misgavage. Deaths of HD animals, however, are not

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[14]Exception: weights of gravid uteri were noted; these did not differ among control and treated dams.

associated with necropsy findings that offer any explanation. Available histopathology is limited to thoracic organs, and reported findings do not reflect lethal, drug-related lesions.

The HD group likewise suffered a loss of body weight, accompanied by the failure to eat (80-90% reduction in chow consumption). This question arises: If this failure to thrive primary? or secondary to some known risedronate toxicity, e.g.gastritis, acute renal insufficiency or hemorrhage? In any event, there are no teratogenicity data available from progeny in this group.

Reflecting mortality data, as well as the occurrences of 1 premature delivery and 1 abortion in the MD group, there was a dose-dependent reduction in the percent of does that carried pregnancies to term: calculations for control, LD, MD and HD were 100%, 85%, 71% and 0%, respectively.

There were no other findings of any significance (statistical or biological) in this study.

Each of these four studies investigating risedronate toxicity clearly demonstrates reproductive consequences, whether drug exposure was pre-conceptional, gestational or throughout gestation and lactation. Unfortunately, interpretation of these findings is difficult for myriad reasons, discussed below. (All references are to rat findings except where the rabbit study is specified.)

1). It is likely that certain of the doses employed in these studies were excessive. Thus, resultant systemic toxicity confounds the examination of primary reproductive toxicities. Preliminary dose-ranging studies submitted in support of revised two-year toxicity and carcinogenicity studies (see Amendment 178) suggest that the MTD for oral risedronate administration is 16 mg/kg/day by gavage when doses are given for 13 weeks. Higher doses in these studies were associated with increased mortality; hematologic sequellae; hypocalcemia and hypophosphatemia; gastrointestinal lesions; and histopathology of kidney, liver, gut, testis, seminal vesicle, bone and tooth.

The findings reported in studies of risedronate reproductive toxicity confirm and extend these dose-ranging data. The rank orders of average daily risedronate doses in these studies are listed below, for males and females, in Tables 22 and 23, respectively. (The MTD of 16 mg/kg/day x 90 days determined for carcinogenicity studies represents a total body burden of ~1460 mg/kg.)

There are four cogent reasons to consider total body burdens exceeding that associated with the 13-week MTD to be dose-limiting in assessment of reproductive effects:

a). there were excessive numbers of deaths in both males[15] and females at these levels, and those in females from the Segment I study all occurred independently of drug-induced peri-parturient mortality;

b). morbidities associated with these groups frequently exhibited an all-or-none character with regard to incidence and intensity - i.e., within a particular study, gradations of response were not generally observed at lower doses;

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[15]Note that dose-limiting effects in males are related to cumulative drug exposure: the initial dose in the 3-generation study, 80 mg/kg/day, is ½ that used initially in Study 995.09.00-AA; and the duration to mortality-related dose reduction in this study is ~ twice that of Study 995.09.00-AA.

c). the numbers of females in these groups actually pregnant at scheduled laparotomy or delivery were insufficient to assess the effects of treatment on parameters of interest; and

d). the number of F_1 litters available for examination in these groups were inadequate to assess this aspect of reproductive toxicity.

Likewise, in the absence of confirmatory dose-ranging data, it is nonetheless clear that the HD in the Segment II (rabbit) study (50 mg/kg/day from gestational days 6-18) is excessive.

TABLE 22
RANK ORDER OF DOSES IN STUDIES OF MALE REPRODUCTIVE TOXICITY

STUDY	CUM DOSE to MATING	AVG DAILY DOSE	BASIS OF CALCULATION
SEGMENT I (HD)	5920 mg/kg	100 mg/kg/day	14 days @ 160 mg/kg/day + ~46 days @ 80 mg/kg/day
3 - GENERATION (HD)	4600 mg/kg	58 mg/kg/day	35 days @ 80 mg/kg/day + ~45 days @ 40 mg/kg/day
SEGMENT I (UMD)	2400 mg/kg	40 mg/kg/day	~60 days @ 40 mg/kg/day
3 - GENERATION (MD)	1280 mg/kg	16 mg/kg/day	~80 days @ 16 mg/kg/day
SEGMENT I (LMD)	600 mg/kg	10 mg/kg/day	~60 days @ 10 mg/kg/day
3 - GENERATION (LD)	256 mg/kg	3.2 mg/kg/day	~80 days @ 3.2 mg/kg/day
SEGMENT I (LD)	150 mg/kg	2.5 mg/kg/day	~60 days @ 2.5 mg/kg/day

TABLE 23 RAT

RANK ORDER OF DOSES IN STUDIES OF FEMALE REPRODUCTIVE TOXICITY

STUDY	CUM DOSE to GEST DAY 20	AVG DAILY DOSE	BASIS OF CALCULATION
SEGMENT I (HD)	2080 mg/kg	95 mg/kg/day	4 days @ 160 mg/kg/day + ~18 days @ 80 mg/kg/day
SEGMENT 1 (UMD)	840 mg/kg	40 mg/kg/day	~21 days @ 40 mg/kg/day
SEGMENT II (HD)	880 mg/kg	80 mg/kg/day	11 days @ 80 mg/kg/day
3-GENERATION (HD)	560 mg/kg	16 mg/kg/day	~35 days @ 16 mg/kg/day
3 - GENERATION (MD)	248 mg/kg	7.1 mg/kg/dy	~35 days @ 7.1mg/kg/day
SEGMENT I (LMD)	210 mg/kg	10 mg/kg/day	~21 days @ 10 mg/kg/day
SEGMENT II (MD)	176 mg/kg	16 mg/kg/day	11 days @ 16 mg/kg/day
3 - GENERATION (LD)	112 mg/kg	3.2 mg/kg/day	~35 days @ 3.2 mg/kg/day
SEGMENT I (LD)	52.5 mg/kg	2.5 mg/kg/day	~21 days @ 2.5 mg/kg/day

2). Studies suffer from a failure in experimental follow-up. Since treated males were mated to treated females in both Segment I and 3-generation studies, the observation of drug-related effects in both sexes necessitates satellite classes of mating pairs comprised of control animals of one sex mated to treated animals of the other. Presently, there are no means to separate apparent drug-induced loss of fertility in males from the total drug effect on the maternal-fetal unit in pre-implantation stages of pregnancy, as measured by the endpoint of reduced pregnancy numbers at day 20. Available data do not quantitate sperm numbers in either ejaculate or cervical mucus; nor are there any descriptions of morphology. Furthermore, there are no available data describing testicular and epididymal histology in males receiving < 40 mg/kg/day. Since it is necessary to severely reduce the population of viable spermatozoa, or to elicit extensive morphological or functional damage in order to alter a measure so gross as the fertility index, it is unclear whether the effects on male reproductive parameters contribute to the observed reduction in fertility.[16] Corroborative data might include the number of cohabitations required for confirmation of copulation; as well as a characterization of sperm viability, or some information on staging. These were not reported. Additional useful data would include effects on gamete maturation in females. N.B. Subsequent Segment II studies, which couple dams treated from gestational days 7-17 with untreated males, do not remedy this deficiency in a way that permits the interpretation of either Segment I or Segment III effects.

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[16]Ecobichon D.J. The Basis of Toxicity Testing. Boca Raton: CRC Press, 1992.

3). The sponsor consistently qualifies rodent findings regarding the numbers of resorptions and implantations with the statement, "*Based upon the number of gravid animals per dose group, ...* ". This is disingenuous, since animals that were not gravid were not examined in any of the 3 rat studies to determine numbers of implantations and early or late resorptions. Additionally, while data for numbers of corpora lutea in dams determined not to be gravid on Day 20 are available in the Segment I study, they are not provided for the other two studies.

The integration of results from the three rat reproductive studies suggests that **there is an element of all-or-none embryonic wastage that is drug related.**[17] **The failure of the sponsor to account for findings in non-gravid rats prevents the examination of this phenomenon.**[18] Substantial numbers of animals are involved. The tallies of affected dams are reproduced below in Table 24.

TABLE 24
Sample Sizes of Rat Dams with Positive Vaginal Signs of Copulation that were Not Gravid at Laparotomy or Delivery

Study	Control	LD	LMD	MD	UMD	HD
Segment I	0/23	2/22	3/24	—	4/22	16/21
3-Generation	2/35	3/35	—	2/35	—	10/32
Segment II	2/36	3/36	—	3/36	—	7/36

The occurrence of massive intrauterine demise suggests that the cause originates in maternal physiology. It is known that calcium is critical to implantation and later gestational events.[19] Since failure of pregnancy progression was more problemic in dams with greater risedronate body burdens (i.e., not randomly distributed among all groups), one logical postulate is that risedronate treatment prevented the mobilization of bone Ca^{++} for pregnancy maintenance.

4). The hypothesis of hypocalcemia-induced uterine inertia as the source of maternal peri-parturient mortality is intuitively attractive, but unsupported by available data. Serum Ca^{++} data from the 3-generation do not even show an *association* between serum calcium and mortality, much less *causality*. This is because no data were obtained from dams permitted to deliver; thus, there is no way to ascertain whether there are significant differences between serum Ca^{++} levels in dams that survived parturition, and those that did not. Specifically, the ranges of values in each treatment group suggests that one or more females at each dosage level had normal serum Ca^{++} levels.

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[17]Particularly the Segment II study, in which untreated males served as sires, and treatment of dams did not begin until gestational day 7

[18]While the sponsor indicated in Methods that these observations were made, the results were not included in these analyses.

[19]Damsky C, Sutherland A and Fisher S. Extracellular matrix 5: Adhesive interactions in early mammalian embryogenesis, implantation and placentation. FASEB J, 7:1320-9, 1993.

5). Neonatal (F_1) mortality data in both the 3-generation and the Segment II studies are difficult to interpret, since the samples are clearly too small when the litter size is [correctly] designated as the unit of evaluation. The sponsor has summarized a number of these findings within the submission: those for the 3-generation study may be found in Table 38 (pg 221); while those for the Segment II experiments are in Table 21 (Pg 98).

Unfortunately, population mortality data were difficult to extract from these tabulations. I have normalized data within each treatment group for the numbers of viable fetuses and subsequently calculated the percent of live births; the percent of deaths from days 0-4 prior to culling; and the percent of deaths from days 5-21 after culling. The results are presented below in Tables 25 and 26.[20] In the absence of sufficient sample sizes when indexed by litter, these data provide a first approximation of the neonatal mortality that might be attributed to risedronate.

TABLE 25
Neonatal Mortality Associated with Pre- and Postnatal Risedronate Exposure

	Control	3.2 mg/kg/day	7.1 mg/kg/day	16 mg/kg/day
# Delivered	175	84	72	37
% Live Births	**99**	**86**	**94**	**81**
% Deaths Days 0-4	**4**	**22**	**7**	**19**
% Deaths Days 5-21[21]	**9**	**0**	**12**	**0**
Total % Deaths	**13**	**22**	**19**	**19**

[20] Note that data from control dam #3663 were excluded; she was clearly dystocic, and died shortly thereafter.

[21] Calculation is based on the number of pups present after culling.

TABLE 26
Neonatal Mortality Associated with Prenatal Risedronate Exposure During the Period of Organogenesis

	Control	3.2 mg/kg/day	16 mg/kg/day	80 mg/kg/day
# Delivered	167	140	92	52
% Live Births	100	100	98	97
% Deaths Days 0-4	0	1.4	3.3	23*
% Deaths Days 5-21[22]	3.4	0	9.4	18.5
Total % Deaths	3.4	1.4	12.7	41.5

While few of these findings were statistically significant, the there are clear trends toward increased mortality with obvious biological significance; the lack of statistical power is probably due to the small sample sizes. Specifically, it is likely that the observed neonatal mortalities in the 3 - generation study at all 3 doses, and in the Segment II study in the mid- and high-dose groups, are excessive. Furthermore, the Sponsor noted that all pups in the Segment II high-dose group were either found dead or euthanized between Days 25 and 27; thus, no pups from this group were mated.

6). **The failure to observe reproductive consequences in the 3-generation and Segment II studies for the F_1 generation is not, in itself, proof of no effect.** Sample sizes were simply too small to rule out F_1 reproductive consequences. (Recall that the numbers of F_0 litters from which F_1 parents were selected was severely reduced, due to the massive numbers of deaths among F_0 dams.)

7). **There are insufficient numbers of fetuses to permit detection of uncommon malformations.** The sponsor has noted in Segment II and III studies that there are no statistically significant increases in the incidences of malformations; and concluded that risedronate is not a teratogen. However, the recurrence of cleft palate in pups of risedronate-treated dams is particularly troubling. The mean incidence of spontaneous cleft palate in Sprague-Dawley pups, derived from examinations of historical control data in several laboratories, was reported to be ~ 0.03%.[22] Since the mean number of fetuses/group in these studies is generally ~ 300, the expected spontaneous frequency of cleft palate in each group would be ~0.09. Extrapolating to an expected frequency of one observation, this indicates that **≥12 such groups of 300 would need to be examined before a single occurrence of spontaneous cleft palate is observed.** Nonetheless, 3 drug-treated fetuses in 3 litters (one low-dose and two mid-dose) were observed in the Segment III study, and two drug-treated

[22]Morita H, et.al.. Spontaneous malformations in laboratory animals: frequency of external, internal and skeletal malformations in rats, rabbits and mice. Cong Anom, 27: 147-206, 1987.

fetuses from two litters (both low-dose) were observed in the Segment II study. The likelihood of this occurring by chance is very small.

8).Changes in weight gain and food consumption reported in the Segment I study are not reliable. It was learned at scheduled laparotomy that a number of females weighed on days 7 and 14 were not actually pregnant. The weight gain determinations of these non-pregnant animals were not removed from consideration for group means and statistical analyses. Because no histology was done to determine whether there were peri-implantation losses in non-gravid females, it is impossible to know how many animals were actually pregnant on day 7. Weight gains in non-pregnant females might be expected to be less than those in gravid females, and could be confounding. Thus, it is recommended that all non-pregnant animals be excluded from the calculation of descriptive statistics and second-order analyses. Similarly, the food consumption data of non-pregnant animals were not removed from consideration for group means and statistical analyses; those data corresponding to non-gravid females should be excluded, and statistical measures recalculated.

Body weight data from the Segment II and 3-generation studies were more compelling, although no biologically significant changes were observed in these parameters in females relative to controls.

Note, too, that food consumption in both control and drug-treated groups was in the Segment I study was 16.72 ± 0.303 g (S.D.) on day 14 of gestation. The intake of the the gravid females Sprague-Dawley rat fed *ad libitum* throughout the course of a 24-hr period is generally greater [23]; and, in fact, was daily in the Segment II and 3-generation studies. This could have had nutritional and metabolic consequences for the pregnancy in the Segment I study.

Safety Margin. The rat NOAEL in males for risedronate is 3.2 mg/kg/day (derived from conditions and parameters of the 3-generation study). This yields a safety margin of 1.02.[24] There was no NOAEL in females.

There was no NOAEL in female rabbits.

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Recommendations:

1. That data from the following studies be disregarded: Segment I, male and female data derived from HD treatment, as well as male data from UMD treatment; 3-generation, male data derived from HD treatment; Segment II (rabbit), HD data. These doses were sufficiently toxic that the overall health of the animal is in question. Reproductive findings in this setting may simply be secondary to gross alterations in physiology.

2. That descriptive statistics and second-order analyses of weight gains and food consumption in females in Study 995.09.00-AA (General Fertility and Reproductive Performance Study Using NE-58095 in Rats) be re-calculated, excluding all animals determined not to be pregnant on day

[23]Baker H.J., Lindsey J.R. and Weisbroth S.H. The Laboratory Rat, Vol 1, Biology and Diseases. New York: Academic Press, Inc., 1979.

[24]Calculations are based on the maximum clinical dose of 30 mg/day employed in the treatment of Paget's; and are corrected for differences in rat and human body surface areas.

20. Furthermore, the Sponsor is requested to provide data documenting sperm viability/motility and maturation, and some analysis of sperm morphology; as well as the potential for reversibility of findings in UMD treated males in this study.

3. That satellite studies be conducted for 995.09.00-AA and 995.09.00-AB (Study of Fertility and General Reproductive Performance Using NE-58095 in Rats...), examining the outcomes of untreated members of one sex mated with treated animals of the opposite sex. This, taken together with results reported here, permits evaluation of the respective contributions of risedronate effects in males and females on fertility, as assessed by reproductive outcome. Protocols should be submitted to the Division for evaluation prior to study initiation.

4. That cleft palate findings in Studies 995.09.00-AB and 995.09.00-AC (Teratology Study Using NE-58095 in Rats - Segment II) be subjected to further statistical analysis. Minimally, for each study a 3x2 Chi Square analysis should be performed on these data, to evaluate whether historical spontaneous frequency, the frequency in controls and the frequency at a particular dose level differ statistically. Optimally, meta-analyses should be performed on the combined studies to determine the probability that the total incidence of cleft palate in drug-treated fetuses is due to random variation.

5. That the Investigator's Brochure and the Informed Consent be amended to reflect that risedronate, like other bisphosponates, causes fatal dystocia in pregnant rat dams.

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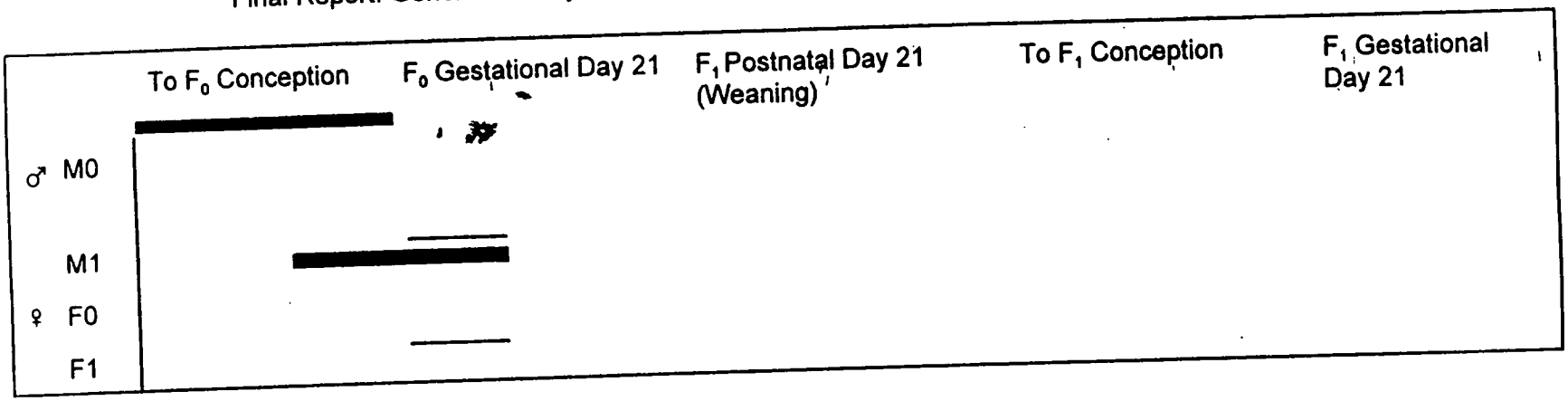
APPENDIX I

Dosing Regimen for 995.09.00-AA

Final Report: General Fertility and Reproductive Performance Study Using NE-58095 in Rats



LEGEND

Direct exposure of animal to drug, via gavage administration

Indirect exposure of fetus via maternal exposure

EXPERIMENTAL PROTOCOL:

Dosing as per above, and with the following doses (mg/kg/day):

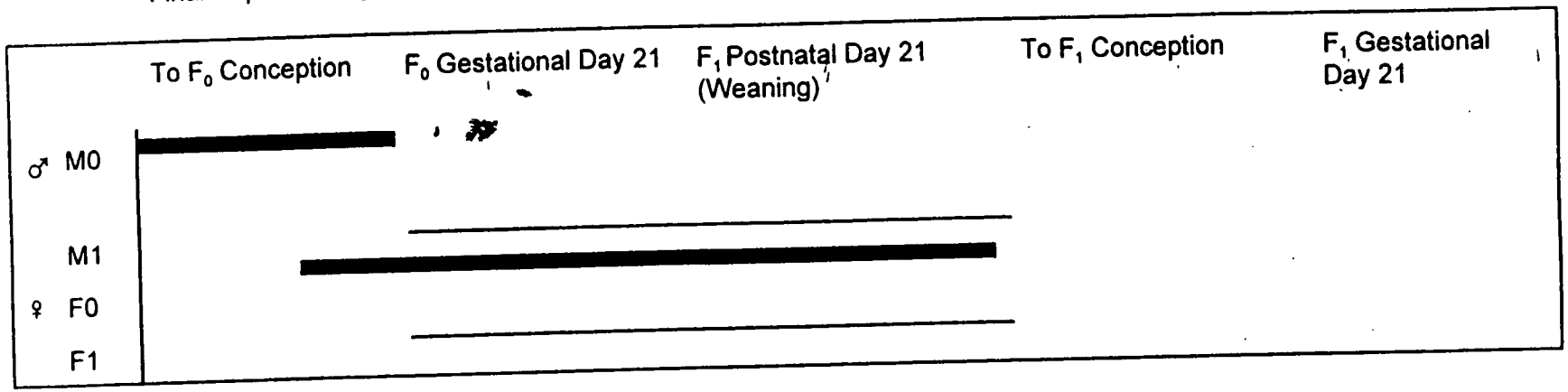
	Low	Med1	Med2	High
♂	- -	10	40	
♀		10	40	- -

Males got drug for at least 60 days prior to mating; tx begun with 160 mg/kg/day and reduced to 80 mg/kg/day on Tx Day16. Females got drug for at least 14 days prior to mating: tx begun with 160 mg/kg/day and reduced to 80 mg/kg/day on Tx day 5. All dosing stopped at conception for males and gestational day 7 for females.

APPENDIX II

Dosing Regimen for 995.09.00-AB

Final Report: Study of Fertility and General Reproductive Performance Using NE-58095 in Rats - Segment I



LEGEND

Direct exposure of animal to drug, via gavage administration

Indirect exposure of fetus/neonate via maternal exposure

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EXPERIMENTAL PROTOCOL:

Dosing as per schedule above, with doses listed in Table 3. All males were treated for at least 80 days prior to mating. In F_0 high-dose males, treatment was begun with 80 mg/kg/day and reduced to 40 mg/kg/day on Tx Day 36. All dosing stopped at conception for males. All females received treatment for at least 15 days prior to mating. This was continued throughout gestation, as well as through lactational day 21 in females selected to nurse and rear their F_1 offspring.

♂	0	3.2	16	80/40
♀	0	3.2 35	16	16

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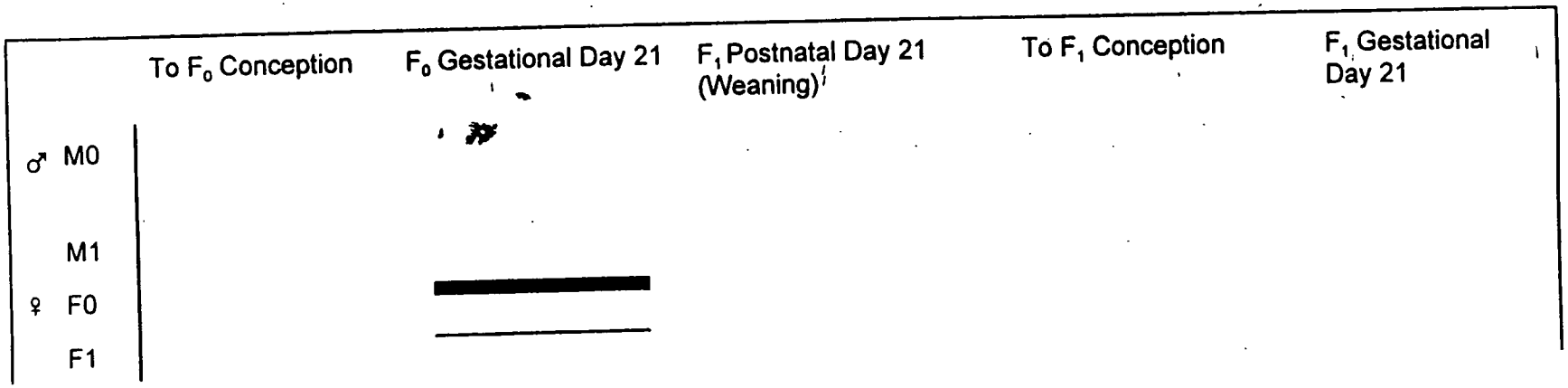
APPENDIX III

Dosing Regimen for 995.09.00-AC
Teratology Study Using NE-58095 in Rats - Segment II



LEGEND

Direct exposure of animal to drug, via gavage administration

Indirect exposure of fetus/neonate via maternal exposure

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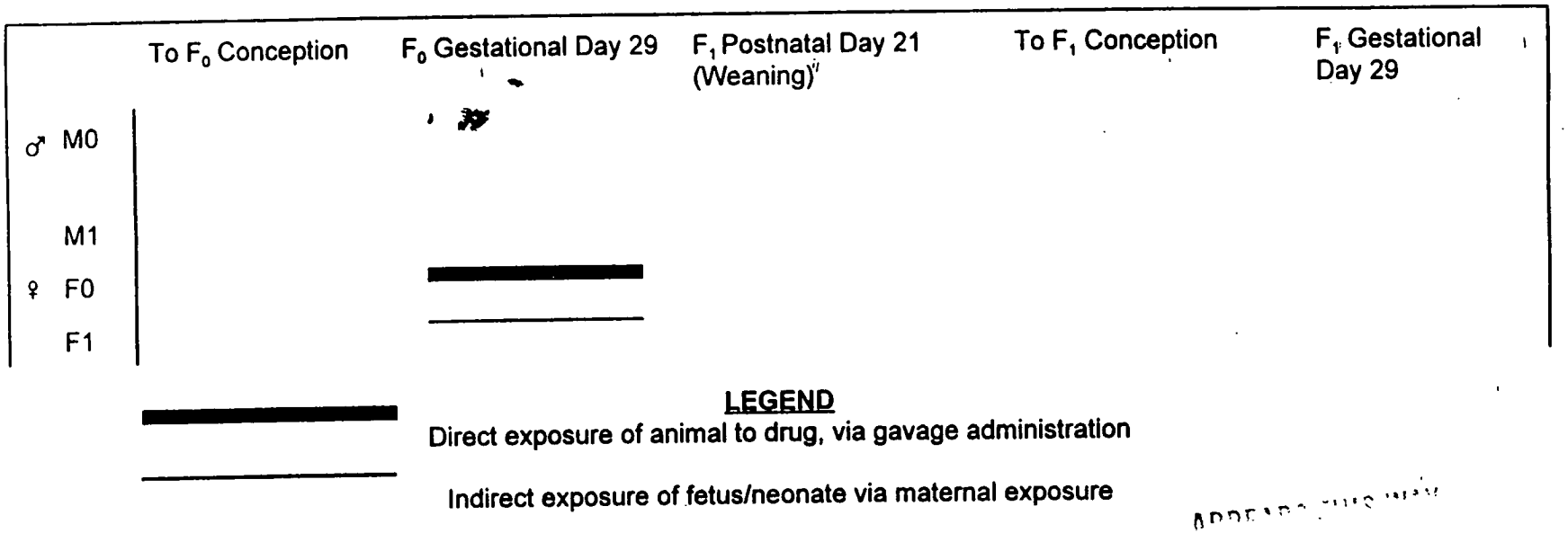
EXPERIMENTAL PROTOCOL:

Control F_0 males were mated to control Fo females (36/sex); or control F_0 males were mated to Tx F_0 females (36 each/sex/group) F_0 females were dosed as per above (days 7-17), and with the following doses (mg/kg/day):

Low	Intermediate	High
	16	

At gestational day 20, 24 dams/group were randomly chosen for C-section. The remainder were permitted to deliver; within this population, ~33% peri-parutrient mortality was observed in intermediate and high-dose groups. Four F_1 pups of each sex from each treatment group were chosen (when possible) for development and behavioral testing. Two other pups from each litter's F_1 generation were retained as parents for the F_2 generation. F_2 females were mated to F_2 males within a given dose level, and females were laparotomized at day 20.

APPENDIX IV
Dosing Regimen for 995.09.00-AD
Teratology Study Using NE-58095 in Rabbits - Segment II



EXPERIMENTAL PROTOCOL:

A cohort of 14 untreated males was used for artificial insemination of control or treated females (18/group). Females were dosed as per above (days 6-18), and with the following doses (mg/kg/day):

Low	Intermediate	High
	10	

A number of females were found dead or euthanized during the course of the study. At gestational day 29, surviving does were sacrificed and a C-section was performed.

Original NDA# 20-835 Initial Labeling Revisions 01/09/98

Drug:	NE-5095
Name:	Risedronate sodium (Actonel).
NDA SUBMITTED:	March 31, 1997
NDA RECEIVED:	April 3,1997
NDA #:	20-835
Sponsor:	Procter & Gamble Pharmaceuticals Sharon Woods Technical Center 11450 Grooms Road Cincinnati, OH 45242-1434
Category:	Bisphosphonate
Indication:	Paget's disease of bone.
Proposed clinical dose:	1 x 30 mg capsule/day for 2 months (18.5 mg/m^2)
Related Submissions:	Efficacy Supplement for Osteoporosis (expected in 1998).
Medical Officer:	G. Troendle/S. Dutta
Chemist:	S. Markofsky
Pharmacologist:	D. Coleman/G. Kuijpers
C. S. O.:	R. Hedin

Following are:
The sponsor's original label.
A revised label.

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Prepared by:

/S/
Gemma Kuijpers, Ph.D.
Pharmacologist

/S/
Daniel T. Coleman, Ph.D.
Pharmacologist.

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13 Page(s) Redacted

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